[0029] In another embodiment, polypeptides may be obtained from the sample by methods known to those of skill in the art. For example, gross preparations of cells obtained using the non-invasive techniques of the invention contain polypeptides. Alternatively, the polypeptides may be further isolated or purified using conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies. The polypeptides may then be characterized to indicate the presence of a dermatatic reaction.

[0030] In one embodiment, the invention provides a method for distinguishing an irritant reaction from an allergic reaction in a skin sample by detecting a polynucleotide encoding a cytokine. The relative quantity of certain cytokines with respect to a normal or standard tissue sample distinguishes the type of reaction and/or the reactions severity.

[0031] While existing clinical tests may not be able to distinguish an irritant reaction from an allergic reaction in the tissue, the non-invasive method of the present invention is capable of distinguishing between the two reactions by their relative cytokine expression profiles. Irritant contact dermatitis can be distinguished from allergic contact dermatitis by the presence or absence of a polynucleotide encoding a cytokine or the cytokine polypeptide.

[0032] For example, in the present invention, cells from ICD had undetectable levels of polynucleotide encoding IL-4 compared with polynucleotides from cells of ACD lesions according to the method used. Consequently, the process may employ, for example, DNA or RNA, including messenger RNA (mRNA), isolated from a tissue. The DNA or RNA may be single stranded or double stranded. When RNA is obtained, enzymes and conditions optimal for reverse transcribing the template to DNA well known in the art can be used. Alternatively, the RNA can be subjected to RNAse protection assays. A DNA-RNA hybrid that contains one strand of each may also be used. A mixture of polynucleotides may also be employed, or the polynucleotides produced in a previous amplification reaction, using the same or different primers may be so used. In the instance where the polynucleotide sequence is to be amplified the polynucleotide sequence may be a fraction of a larger molecule or can be present initially as a discrete molecule, such that the specific sequence is the entire nucleic acid. It is not necessary that the sequence to be amplified be present initially in a pure form; it may be a minor fraction of a complex mixture, such as contained in whole human DNA.

[0033] In addition, RNAse protection assays may be used if RNA is the polynucleotide obtained from the sample. In this procedure, a labeled antisense RNA probe is hybridized to the complementary polynucleotide in the sample. The remaining unhybridized single-stranded probe is degraded by ribonuclease treatment. The hybridized, double stranded probe is protected from RNAse digestion. After an appropriate time, the products of the digestion reaction are collected and analyzed on a gel (see for example Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, section 4.7.1 (1987)). As used herein, "RNA probe" refers to a ribonucleotide capable of hybridizing to RNA in a sample of interest. Those skilled in the art will be able to identify and modify the RNAse protection assay specific to the polynucleotide to be measured, for example, probe specificity may be altered, hybridization temperatures, quantity of nucleic acid etc. Additionally, a number of commercial kits are available, for example, RiboQuantTM Multi-Probe RNAse Protection Assay System (Pharmingen, Inc., San Diego, CA).

[0034] In another embodiment, the polynucleotide in the sample may be analyzed by Northern or Southern blot. In this technique the polynucleotides are separated on a gel and then probed with a complementary polynucleotide to the sequence of interest. For example, RNA is separated on a gel transferred to nitrocellulose and probed with complementary DNA to the sequence of interest. The complementary probe may be labeled radioactively, chemically etc. Hybridization of the probe is indicative of the presence of the polynucleotide of interest.

[0035] Detection of a polynucleotide encoding a cytokine may be performed by standard methods such as size fractionating the nucleic acid. Methods of size fractionating the DNA and RNA are well known to those of skill in the art, such as by gel electrophoresis, including polyacrylamide gel electrophoresis (PAGE). For example, the gel may be a denaturing 7 M or 8 M urea-polyacrylamide-formamide gel. Size fractionating the nucleic acid may also be accomplished by chromatographic methods known to those of skill in the art.

[0036] The detection of polynucleotides may optionally be performed by using radioactively labeled probes. Any radioactive label may be employed which provides an adequate signal. Other labels include ligands, colored dyes, and fluorescent molecules, which can serve as a specific binding pair member for a labeled ligand, and the like. The labeled preparations are

used to probe for a polynucleotide by the Southern or Northern hybridization techniques, for example. Nucleotides obtained from samples are transferred to filters that bind polynucleotides. After exposure to the labeled polynucleotide probe, which will hybridize to nucleotide fragments containing target nucleic acid sequences, the binding of the radioactive probe to target nucleic acid fragments is identified by autoradiography (see *Genetic Engineering,* 1 ed. Robert Williamson, Academic Press (1981), pp. 72-81). The particular hybridization technique is not essential to the invention. Hybridization techniques are well known or easily ascertained by one of ordinary skill in the art. As improvements are made in hybridization techniques, they can readily be applied in the method of the invention.

[0037] The polynucleotides encoding the desired polypeptide may be amplified before detecting. The term "amplified" refers to the process of making multiple copies of the nucleic acid from a single polynucleotide molecule. The amplification of polynucleotides can be carried out *in vitro* by biochemical processes known to those of skill in the art. The amplification agent may be any compound or system that will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, *E. coli* DNA polymerase I, Taq polymerase, Klenow fragment of *E. coli* DNA polymerase I, T4 DNA polymerase, other available DNA polymerases, polymerase muteins, reverse transcriptase, ligase, and other enzymes, including heat-stable enzymes (*i.e.*, those enzymes that perform primer extension after being subjected to temperatures sufficiently elevated to cause denaturation). Suitable enzymes will facilitate combination of the nucleotides in the proper manner to form the primer extension products that are complementary to each mutant nucleotide strand. Generally, the synthesis will be initiated at the 3'-end of each primer and proceed in the 5'-direction along the template strand, until synthesis terminates, producing molecules of different lengths. There may be amplification agents, however, that initiate synthesis at the 5'-end and proceed in the other direction, using the same process as described above. In any event, the method of the invention is not to be limited to the embodiments of amplification described herein.

[0038] One method of *in vitro* amplification which can be used according to this invention is the polymerase chain reaction (PCR) described in U.S. Patent Nos. 4,683,202 and 4,683,195.